Available online on 25.12.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Elucidating the Structural Requirements of Novel Pazopanib Derivatives towards Tyrosine Kinase Inhibitory Activity through Classical Hansch and *De-Novo* Approach

Nidhi Gupta, Love Soni

School of Pharmacy, Devi Ahilya University, Khandwa Road, Indore, 452001(M.P.), India

Email: gnidhi504@gmail.com

ABSTRACT

Quantitative structure–activity relationship (QSAR) and Molecular modeling study have been performed on a series of novel Pazopanib derivatives by using the Mixed Hansch Fujita-Ban approach and employing AM1 calculations in docking study which give insight into the structural requirements of the derivatives towards inhibitory activity against VEGFR-2, PDGFR-α and c-kit tyrosine kinases enzymes. Evaluation of the predictive ability of the resulting models is carried out by using 'Leave-one-out' (LOO) method of cross validation. There is a remarkable agreement in the results of both the approaches. From the QSAR study it can be inferred that methyl substitution at 5th position of the terminal benzene ring and hydrophobicity play a key role in kinase inhibitory activity. Potential binding sites were elucidated by docking study. Docking simulations was performed through Molegro Virtual Docker (MVD) for lead optimization of compound as protein tyrosine kinase inhibitors.

Cite this article as: Gupta N, Soni L, Elucidating the Structural Requirements of Novel Pazopanib Derivatives towards Tyrosine Kinase Inhibitory Activity through Classical Hansch and De-Novo Approach, J. of Drug Delivery & Therapeutics. 2017; 7(7):161-164

INTRODUCTION:

Cancer is the second leading cause of death in the western world. Despite advances in diagnosis and treatment, overall survival of patients still remains poor. This has improved survival in several types of solid tumors, but treatment-related toxicity and emergence of drug resistance have been the major cause of morbidity and mortality. Hence, there is an urgent need to develop newer more effective therapy to improve patient outcomes [1].

Tyrosine kinases (TKs) represent a major subclass of protein kinases, which play a pivotal role for intracellular signal transduction. Crucial cellular processes are regulated by TK signaling, such as adhesion, proliferation, migration, invasion, differentiation, metabolism, angiogenesis, survival and apoptotic cell death [2]. Tyrosine kinase is an enzyme that transfers a phosphate group from ATP to a tyrosine residue on specific cellular proteins. Many growth factors such as insulin, epidermal growth factor, and platelet-derived growth factor mediate their effects via receptor, switches on the kinase activity of catalytic domain. This signaling cascade is altered in cancer cell, which cause over expression of TK receptors. TKs generally activate downstream target proteins through phosphorylation or provide binding sites for protein–protein interactions [3]. Pazopanib (Votrient, GW786034) is a novel multi-targeted receptor tyrosine kinase inhibitor, having both anti-proliferative and anti-angiogenic properties, targeting the vascular endothelial growth factor receptor (VEGFR-1, -2 and -3), platelet-derived growth factor receptor (PDGFR-α and -β), and c-kit. It was first approved by Food Drug Administration (FDA) as an agent to treat metastatic renal cell carcinoma in 2009, and again approved by FDA in 2012 to treat soft tissue sarcoma. Clinical experience with Pazopanib demonstrates the advantages of broad-spectrum anticancer potency and less prone to resistance[4].

The aim of this study is to explore the structural requirements of pazopanib derivatives for inhibiting the tyrosine kinase enzyme activity by employing two – dimensional (2D) QSAR approach. Further docking study has been performed on this series by using Molegro Virtual Docker (MVD) 1.2 software. This study gives the idea for rational design of tyrosine kinase inhibitors which will exhibit greater therapeutic efficacy and safety.

MATERIALS AND METHODS:

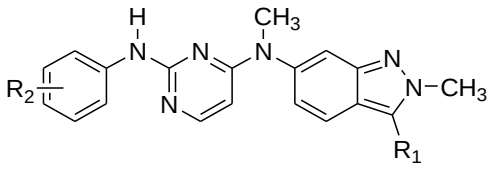
Data Set and Molecular Modeling

Thirteen compounds were taken for the QSAR and docking study as tyrosine kinase inhibitors from the previously reported literature[5]. The biological activity

data (IC_{50} in nM) was converted into negative logarithmic dose ($-logIC_{50}$ or pIC_{50} in mole) to reduce the skewness of data taken as dependent variable, which exhibit the correlation with independent variable or descriptors listed in (table 1).

$$pIC_{50} = -\log_{10} IC_{50} \quad (1)$$

Where, IC_{50} is the nano molar concentration of the kinase inhibitors producing 50% inhibition. Initially series was subjected to QSAR analysis via De Novo contribution of substituent to the activity of molecule by employing Mixed Hansch Fujita-Ban approach. Here physicochemical descriptors and indicator variables which are used in Hansch-FB analysis. Molecular modeling study was carried out via Chem Office Ultra Version 8.0(Cambridge Soft.Corp.), Molegro virtual Docker (MVD) 1.2 and regression analysis study was done by using VALSTAT software. Sequential Multiple Linear regression (SMLR) analysis was used to generate the QSAR model. Validation is a crucial step in any QSAR modeling method. It is needed to establish the predictiveness of a model on unseen data and it helps to determine the complexity of an equation that the amount of data justifies. Model is validated both internally (least square fit R^2, Leave-one out-cross validation or q^2, γ randomization, boots-trapping r^2_{bs}) and externally (predictive ability of correlation coefficient r^2_{pred}).



7a-m

Table 1: Common Structure and Inhibitory activity data (IC_{50}) of pazopanib analogs as anticancer agents

S.No.	Compd	R1	R2	IC_{50}(nM)			pIC_{50}(M)		
				VEGF R-2	PDGFR-α	C-Kit	VEGFR-2	PDGFR-α	C-Kit
1	7a	CH3	3-F	78	130	102	7.108	6.886	6.991
2	7b	CH3	3-Br	64	97	98	7.194	7.013	7.009
3	7c	CH3	3-Cl	25	85	80	7.602	7.071	7.097
4	7d	CH3	3OCH3	38	96	72	7.420	7.018	7.143
5	7	CH3	3-CH3	42	80	87	7.377	7.097	7.060
6	7f	CH3	3,5-DiMethyl	21	52	40	7.678	7.284	7.398
7	7g	CH3	4-SCH3	51	94	71	7.292	7.027	7.149
8	7h	CH3	4-OCF3	72	104	89	7.143	6.983	7.051
9	7i	H	3-F	93	140	96	7.032	6.854	7.018
10	7j	H	3-Cl	72	95	72	7.143	7.022	7.143
11	7k	H	3-OCH3	108	86	77	6.967	7.066	7.114
12	7l	H	4-OCF3	12	72	83	7.921	7.143	7.081
13	7m	H	3,5-DiMethyl	28	75	61	7.553	7.125	7.215

The docking study was performed to observe the interaction of all compounds with the receptor and to examine the agreement between the docking pattern and predictive activity of the validated pharmacophore. The docking study was carried out on tyrosine kinase enzyme using Molegro virtual docker (MVD) 1.2 software by 64 bit operating system under window 8 with an Intel ®Celeron® Processor N2840. Reported crystal structure of tyrosine kinase enzyme inhibitor was extracted from the Protein Data Bank (PDB Id: 1t46) (http://www.rcsb.org/pdb). Before docking, protein is prepared by using Protein preparation wizard, where water molecule and cofactors are removed from the proteins, optimizing the hydrogen bonds and deleting the ligands present in the crystal structure. For docking, ligand structures were prepared using Cambridge software and were subjected to energy minimization. The active site was generated using grid box. The lowest energy conformation was selected where RMS gradient reaches to 0.01kcal/mol and RMS distance to 0.1A^o and were also subjected to energy minimization.

RESULTS AND DISCUSSION:

Generation of QSAR Model for Hansch-Fujita ban approach

Model-1 (for VEGFR-2 receptor)

BA= [7.527(± 0.247)] +HA [0.469(± 0.691)] +$\mathcal{R}$ [1.131(± 1.089)]

Contribution of parameters to model is HA:$\mathcal{R}$::1:6.168……(1)

n=13, r=0.600, r^2=0.360, r^2adj=0.232, std=0.248, F=2.814

Model-2 (for PDGFR-α receptor)

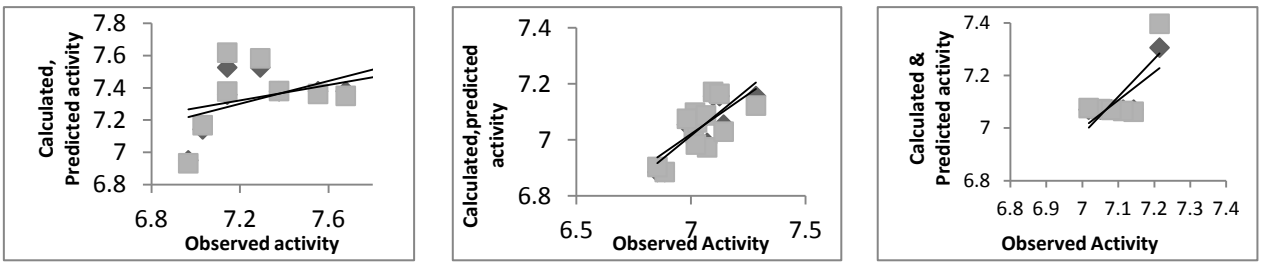
BA= [7.036(± 0.084)] +MR [0.019(± 0.015)] - $\mathcal{F}$ [0.388(± 0.217)]

Contribution of parameters to model is MR:$\mathcal{F}$::1.128:1……(2)

n=13, r=0.818, r^2=0.669, r^2adj=0.603, std=0.069, F=10.117, FIT=1.190,

Bivariant equations were generated for both VEGFR and PDGFR-α receptor, which mediate tyrosine-kinase inhibitiory activity. In model 1, difference between r^2_{adj} and r^2 is less than 0.3, which indicates that model is significant. Internal validity of the model is observed via q^2 or cross validated correlation-coefficient. Model also express S_{PRESS} and S_{DEP} activity. Model 1 suggested that

hydrogen acceptor group should be present in the designed molecule, which contribute positively and favored towards the inhibition. Whereas in model 2, MR contribute positively and field effect contribute negatively to the activity, which suggests that less bulky group is optimum for the activity.



Model-1 **Model-2** **Model-3**

Figure 1: Plot between observed vs calculated and predicted activity of model-1,2,3

Model-3 (for c-Kit receptor)

BA= [7.071(± 0.047)] +R_4SMe [0.078(± 0.156)] +R_5C [0.236(± 0.115)]

Contribution of parameters to model is
$R_4S:R_5C::1:6.022$……(3)

n=13, r=0.827, r^2=0.685, r^2adj=0.622, STD=0.066, F=10.860

This model gives the idea about the substitution at 4 and 5 position in designing the analogues for c-kit receptor. According to the model, at 4th position thiomethyl substitution and 5th position methyl group should be present there for its inhibitory activity.

Table 2: Observed, calculated and predicted IC_{50} (by the LOO method) via Hansch-Fujita ban approach of pazopanib analogs as anticancer agents

Hansch-Fujita- ban analysis											
VEGFR-2 (Model-1)				PDGFR-α (Model-2)				c-Kit (Model-3)			
Compd.	Obs.	Calc.	Pred.$_{(Loo)}$	Compd.	Obs.	Calc.	Pred.$_{(Loo)}$	Compd.	Obs.	Calc.	Pred.$_{(Loo)}$
1	7.108	7.143	7.151	**1**	6.886	6.886	6.886	**1**	6.991	7.071	7.080
2	7.194	7.335	7.348	**2**	7.013	7.033	7.042	**2**	7.009	7.071	7.078
3	7.602	7.358	7.335	**3**	7.071	6.990	6.974	**3**	7.097	7.071	7.068
4	7.420	7.420	-	**4**	7.018	7.084	7.098	**4**	7.143	7.071	7.063
5	7.377	7.380	7.381	**5**	7.097	7.157	7.172	**5**	7.060	7.071	7.072
6	7.678	7.380	7.351	**6**	7.284	7.157	7.123	**6**	7.398	7.307	7.215
7	7.292	7.527	7.584	**7**	7.027	7.055	7.063	**7**	7.149	7.149	-
8	7.143	7.527	7.619	**8**	6.983	7.055	7.076	**8**	7.051	7.071	7.073
9	7.032	7.143	7.169	**9**	6.854	6.886	6.904	**9**	7.018	7.071	7.077
10	7.143	7.358	7.378	**10**	7.022	6.991	6.984	**10**	7.143	7.071	7.063
11	6.967	6.951	6.933	**11**	7.066	7.084	7.088	**11**	7.114	7.071	7.066
12	7.921	7.527	7.433	**12**	7.143	7.055	7.030	**12**	7.081	7.071	7.070
13	7.553	7.380	7.363	**13**	7.125	7.157	7.165	**13**	7.215	7.307	7.398

Docking Studies

Docking study was performed to gain structural insight into the binding mode of most active compound. This study was carried out via Molegro Virtual Docker program on (Pdb Id: 1t46) (fig.2).as tyrosine kinase inhibitors. The best binding model of compound 7f is shown in (fig.3). In the binding model, compound 7f was nicely bound with ATP binding site of kinase inhibitor receptors through hydrophobic, H-bonding and steric interactions. Docking score of compound 7f was found to be -151.594, indicates high binding affinity and better H-Bonding interactions with protein (1t46) residues. From the figure, it was revealed that compound 7f was nicely bound to Asp 810A amino-acid residues by two hydrogen bonds. Among them, One H-bond is formed between amino (-NH-) group of Pyrimdine ring and amino –acid residue of Asp810A. Whereas another –NH- group between phenyl and pyrimidine ing also takes part in H-bonding interaction. Data of kinase inhibition study suggested that, H-bonding interaction plays an important role in its inhibitory activity. Rather than H-bonding interaction, compound 7f also exhibited hydrophobic and steric interaction with Glu640, Val668, Val654 and Leu644 amino-acid residues.

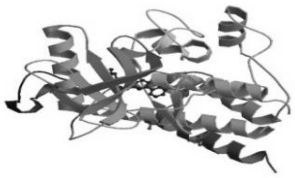


Figure 2 (a) : 3D crystal structure of tyrosine kinase inhibitors pdb code:1t46

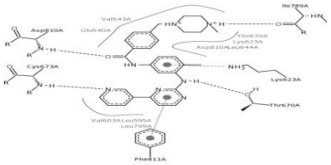


Figure 2(b): 2D view of receptor and their interactions is Shown.

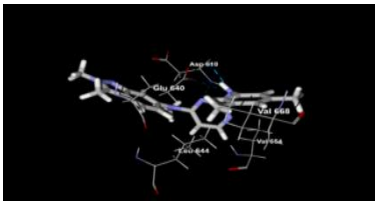


Figure 3: Molegro predicted binding mode of the ligand 7f is shown. Blue colour indicates hydrogen bonding interactions, and red colour shows steric interaction.

CONCLUSION:

In this paper, QSAR and docking study was performed on a series of pazopanib derivatives as anticancer agents, where inhibitory activity was observed against VEGFR-2, PDGFR-α and c-Kit tyrosine-kinase enzyme. QSAR models were developed by using Sequential multiple linear regression analysis. Mixed Hansch Fujita-Ban approach suggested that R2 substituent in pazopanib ring is more favorable for kinase inhibition and lipophilicity also plays crucial role towards its inhibitory activity. Data also suggested that more polar and less bulky group contribute positively at R2 position, whereas R1 position is unsubstituted or substituted by smaller group play an important role to its inhibitory activity. Docking study revealed that H-bond interaction plays significant role in the kinase inhibition activity. On the basis of docking analysis, compound 7f was found most potent compound as tyrosine-kinase inhibitors.

Acknowledgements: Author (NG) is grateful to Department of Science & Technology (DST) - Innovation in Science Pursuit for Inspired Research (INSPIRE) for providing DST-INSPIRE fellowship and also thankful to the Head, department of School of Pharmacy.

REFERENCES:

1. Srinivasan M, Trivedi SG, Tyrosine kinase inhibitors in cancer therapy, Clin Biochem, 2004, 37, 618-635.
2. Levitzki A, Protein Tyrosine Kinase Inhibitors as Novel Therapeutic Agents, P&T, 1999, 82, 231-239.
3. Levitzki A, Gazit A, Tyrosine kinase inhibition: an approach to drug development, Science, 1995, 267, 1782-1788.
4. Bari SB, Adhikari S, Surana SJ, Tyrosine Kinase Receptor Inhibitors: A New Target for Anticancer Drug Development, JPST, 2012, 1, 36-45.
5. Dengke L, Haofei Q, Ligong C, Bingni L, Xinran W, Synthesis and biological evaluation of novel pazopanib derivatives as antitumor agents, BMCL, 2014, 24, 1108-1110.